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Dimensions of elongated mineral particles: a study of more than 570 fibers from more than 90 cases with implications for pathogenicity and classification as asbestiform vs. cleavage fragments

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ABSTRACT

Asbestos is well-recognized as the cause of a variety of disorders of the respiratory tract, including neoplastic as well as non-neoplastic conditions. Fiber dimensions and biopersistence are important determinants of the pathologic response, and analytical electron microscopy is a powerful technique for determining the fiber content of lung tissue samples. For decades our laboratory has examined lung tissue samples counting fibers measuring 5 µm or greater in length. More recent observations have indicated that fibers 10 µm or greater in length are pathogenic, and that a length of 10 µm and diameter less than 1.0 µm are useful features for distinguishing asbestiform fibers from cleavage fragments. We examined more than 570 fibers from more than 90 cases to determine the dimensions of fibers that might be classified as asbestos. The vast majority of fibers classified as amosite or crocidolite met the criteria for length greater than 10 µm and diameter less than 1.0 µm. However, a significant proportion of fibers classified as tremolite, actinolite, or anthophyllite did not meet these criteria. These findings have important implications for the identification and classification of elongated mineral particles, both in terms of pathogenicity as well as classification as asbestiform vs. cleavage fragments.

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Introduction

Asbestos has been implicated in the pathogenesis of a wide variety of neoplastic and non-neoplastic respiratory diseases. The term asbestos refers to both serpentine and amphibole minerals. The former includes chrysotile asbestos and the latter includes five amphibole minerals: amosite, crocidolite, tremolite, actinolite, and anthophyllite.[1] These minerals occur not only as mineral fibers but also as nonasbestiform varieties. An important area of concern is the distinction of true asbestiform minerals from cleavage fragments derived from the nonasbestiform habit.

Asbestos-related diseases tend to follow a dose-response relationship, with the cumulative dose being an important determinant of disease.[2] Since the mid-1970s, analytical electron microscopy has provided a valuable method for determining the cumulative dose of asbestos within the lungs, and in many instances, has greatly improved our understanding of asbestos fiber type, dimensions, and disease.[3] Both analytical transmission and scanning electron microscopy have made valuable contributions in this regard.[2]

For decades it has been recognized that short asbestos fibers, i.e., those less than 5 µm in length, are neither fibrogenic nor carcinogenic in experimental animal models.[4] Our laboratory has been performing fiber analyses of lung tissue samples since 1980, counting only those fibers that are 5 µm or greater in length with aspect ratios of at least 3:1 and roughly parallel sides. However, more recent analyses have indicated that it is likely only fibers that are 10 µm or greater in length that are pathogenic.[5] Furthermore, it has been reported that the best morphologic criteria for distinguishing cleavage fragments from true asbestos fibers is a length of at least 10 µm and a diameter of less than 1.0 µm.[6] This distinction is important since there is no convincing evidence for the

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pathogenicity of cleavage fragments.[7] It has been our impression that this distinction has little effect on the identification of amosite or crocidolite as asbestos but might have a considerable effect on the identification of non-commercial amphiboles, including tremolite, actinolite, and anthophyllite.[2] Since 2012, we have documented the dimensions of fibers meeting our previous criteria for asbestos and include the results of this analysis herein.

Materials and methods

The records of a large database were reviewed to identify cases where a fiber analysis had been performed and dimensions of asbestos fibers recorded on the count sheet. Fibers were identified as asbestos by morphology and elemental composition as determined by energy dispersive x-ray analysis as previously described.[2] In brief, a JEOL JSM 6400 scanning electron microscope equipped with an energy dispersive spectrometer was utilized to examine Nuclepore filters prepared from lung tissue digests. The accelerating voltage was 20 kV and the screening magnification 1300x. A total of 100 fields or 200 fibers were counted, whichever came first. Up to 20 uncoated fibers and 10 asbestos body cores were examined by energy dispersive x-ray analysis. Fibers were classified as amosite, crocidolite, tremolite, actinolite, anthophyllite, or chrysotile based upon morphology and elemental composition, as previously described (Figures 1 and 2).[1,2]

A total of 573 such fibers were identified from 91 cases, including 301 amosite, 84 crocidolite, 78 tremolite, 23 anthophyllite, 22 actinolite, and 31 chrysotile. Thirty-two additional fibers were identified as Libby amphibole (not currently classified as asbestos), a recognized contaminant of vermiculite mined near Libby, MT.[8] For two additional fibers, a distinction between amosite and crocidolite could not be made. Fiber dimensions were measured on screen and recorded on the count sheets. The proportion of fibers exceeding 10 μm in length and with diameters less than 1.0 μm was also determined for each fiber type.

Fibers were not normally distributed, thus the length and diameter data are tabulated by fiber type using the median and range. Generalized repeated measures models (Poisson or lognormal distribution) were used to compare the least squares mean (LSM) estimates for both length

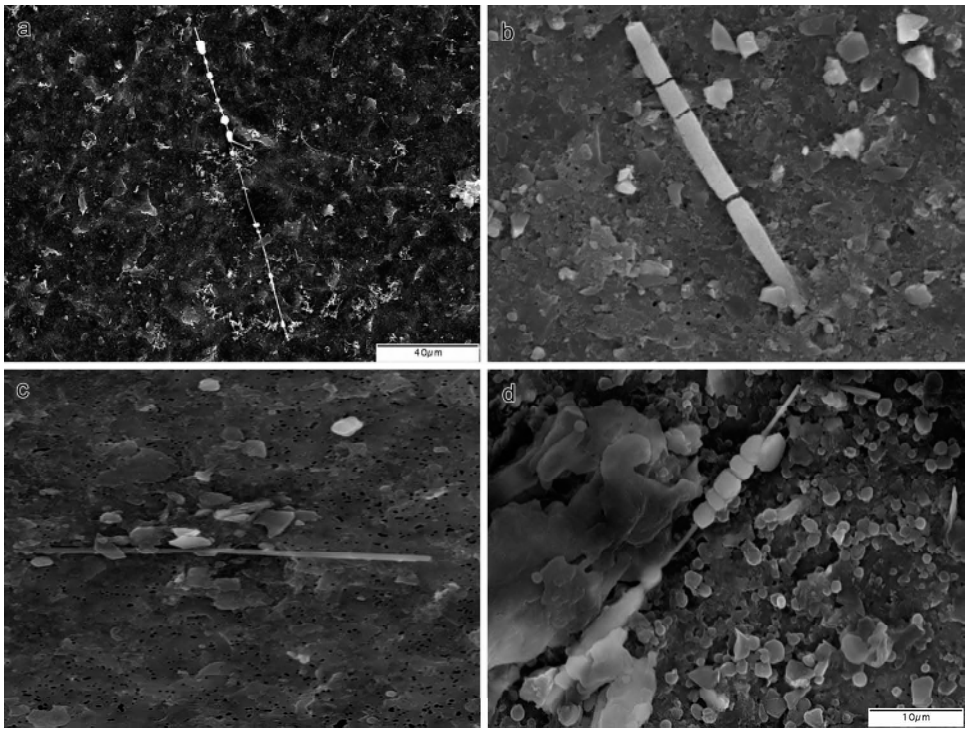

Figure 1. Representative secondary electron images of fibers. (a) Crocidolite fiber from the lung of an insulator; (b) amosite fiber from the lung of an insulator; (c) Libby amphibole from the lung of a resident of Libby, MT; (d) anthophyllite fiber from the lung of chemical plant worker. (a) x2000, (b) x2700, (c) x6000, (d) x5000.

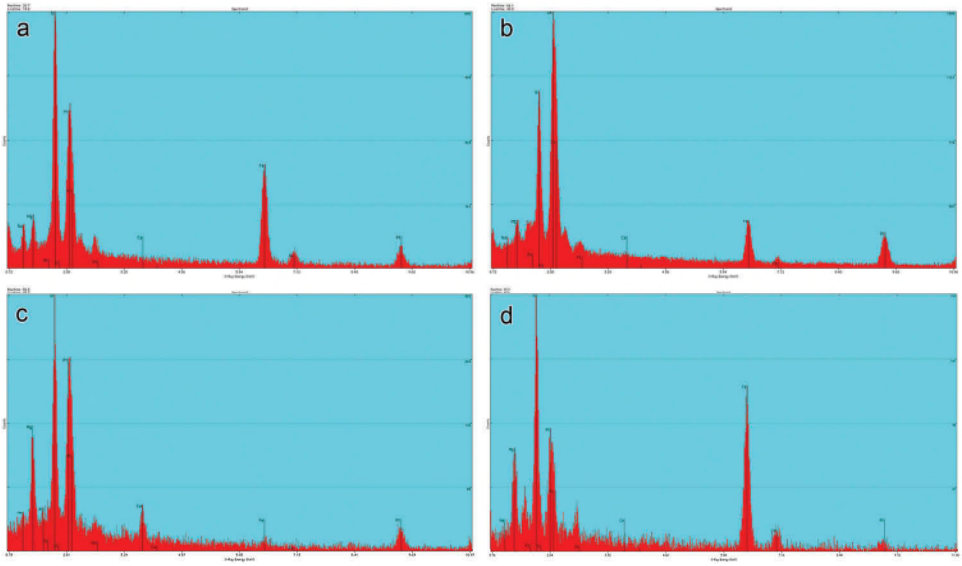

Figure 2. Representative energy dispersive spectra from fibers shown in Figure 1. (a) Crocidolite spectrum with peaks for Na, Mg, Si, and Fe. (b) Amosite spectrum with peaks for Mg, Si, and Fe. (c) Libby amphibole with peaks for Mg, Si, and Ca. Additional small peaks for Na and Fe distinguish Libby amphibole from tremolite. (d) Anthophyllite spectrum with peaks for Mg, Si, and Fe. Additional Pt peak in each spectrum derives from sputter coating.

and diameter between fiber types. Differences between fiber types were computed using the rate ratio from the regression model. All analyses were done using SAS version 9.4 (SAS, Institute, Inc., Cary, NC) and a p-value <0.05 was considered statistically significant.

Results

The median length and diameter values as well as the ranges for each fiber type are indicated in Table 1. Amosite fibers were 1.28 times longer than crocidolite fibers ($p = 0.008$); whereas, crocidolite fibers were 25% thinner than amosite fibers ($p < 0.001$). Amosite fibers were 1.49 times longer ($p = 0.040$) and 49% thinner ($p = 0.001$) than Libby amphiboles. Amosite fibers were 2.34 times longer and 54% thinner than tremolite ($p < 0.001$), whereas Libby amphiboles were 1.66 times longer ($p = 0.003$) but only 12% thinner ($p = 0.250$) than tremolite. Anthophyllite fibers were 1.93 times longer than tremolite ($p < 0.001$) but their diameters were similar ($p = 0.783$). Chrysotile fibers were 1.39 times longer ($p = 0.017$) and 36% thinner ($p = 0.001$) than tremolite. Amosite fibers were 1.42 times longer ($p < 0.001$) and, surprisingly, 31% thinner ($p < 0.001$) than chrysotile. Tremolite and actinolite had similar lengths and diameters ($p = 0.363$ and 0.684, respectively).

Only 9% of amosite fibers and 12% of crocidolite fibers analyzed were less than 10 µm long, and only 1% of either fiber type had a diameter greater than 1.0 µm. Similarly, only 1% of crocidolite fibers and none of the amosite fibers analyzed met the criteria of both length less than 10 µm and diameter greater than 1.0 µm. Thus, our identification of amosite or crocidolite as asbestos (rather than cleavage fragments) is minimally affected by the newly published data. On the other hand, 42% of tremolite, 41% of

Table 1. Dimensional characteristics of 573 Fibers from 91 cases*.

Metrics	Amosite	Crocidolite	Tremolite	Actinolite	Anthophyllite	Chrysotile	LA
N	301	84	78	22	23	31	32
Med L	25	20	10	10.5	23	12	18
Range L	4–220	5–65	4–60	5.0–65.0	6–62	5–100	5–67
Med D	0.40	0.30	1.00	0.90	0.90	0.50	0.85
Range D	0.10–1.20	0.10–1.20	0.25–2.00	0.30–1.90	0.30–2.00	0.15–4.00	0.40–3.00

*Values represent fiber length (L) and diameter (D) in microns, with Med representing the median and the range indicated underneath. LA = Libby amphibole.

actinolite, and 13% of anthophyllite fibers analyzed were less than 10 µm long, whereas 36% of tremolite, 23% of actinolite, and 39% of anthophyllite fibers had a diameter greater than 1.0 µm. Ten percent of tremolite and 14% of actinolite fibers met the criteria of both length less than 10 µm and diameter greater than 1.0 µm and thus were likely cleavage fragments. Approximately 3% of fibers classified as Libby amphiboles met both the length and diameter criteria for cleavage fragments noted above (Table 2).

A total of 108 asbestos bodies were separately analyzed, including 87 amosite, 15 crocidolite, 3 actinolite, 1 tremolite, and 1 anthophyllite. For one additional asbestos body, a distinction between amosite and crocidolite could not be made. In this series, no chrysotile or Libby amphibole asbestos bodies were identified. Asbestos bodies were on average longer than the uncoated fibers (grand median 32 µm with a range of 6 to 220 µm). The median diameter of asbestos bodies was either similar or slightly less than that of the corresponding uncoated fibers (grand median 0.4 µm with a range of 0.1 to 1.2 µm). More than 95% of asbestos bodies analyzed had a commercial amphibole core (mostly amosite with some crocidolite).

Discussion

The findings in our present study indicate that crocidolite fibers are on average thinner than amosite fibers, which in turn are thinner than tremolite or actinolite. Amosite and crocidolite fibers were on average longer than tremolite or actinolite. Interestingly, the median length of anthophyllite fibers was similar to that of amosite and crocidolite, although the former fibers were significantly thicker. These observations are similar to what others have reported with respect to fiber dimensions from human lung tissue samples.[2,9]

Libby amphiboles include a mixture of winchite, richterite, and tremolite.[8] The fibers we analyzed that had an elemental composition indicative of Libby amphibole were significantly longer than tremolite fibers, but of similar thickness. One surprising observation was that our amosite fibers were significantly longer and thinner than the chrysotile fibers that we identified. This is likely due to the fact that chrysotile tends to break down into shorter fibers within the lung, which are not counted by our methodology.[10] In addition, many of the longer chrysotile fibers are too thin to be observed at our screening magnification.[11] Most of the chrysotile fibers that we counted were fiber bundles.

Our findings demonstrate that lack of pathogenicity of fibers less than 10 µm long or likelihood of cleavage fragments for fibers less than 10 µm long and greater than 1.0 µm in diameter has little or no effect on the classification of commercial amphibole fibers using our analytical methodology. On the other hand, both lack of pathogenicity and likelihood of cleavage fragments apply to a significant proportion of non-commercial amphiboles identified using our counting scheme. Therefore, when counted, the dimensions of these non-commercial amphibole fibers need to be recorded.

The demonstration that more than 95% of asbestos bodies form on commercial amphibole cores is consistent with our previously reported observations.[12] Furthermore, the observation that asbestos bodies tend to be longer than uncoated fibers of similar type confirms prior observations in this regard.[13,14] Although in this study we did not find any asbestos bodies with chrysotile or Libby amphibole cores, such asbestos bodies have been observed in previous reports.[12,15]

In conclusion, a significant proportion of non-commercial amphiboles (tremolite, actinolite, or anthophyllite) that meet the criteria of length greater than 5 µm and 3:1 aspect ratio with roughly parallel

Table 2. Percentage of fibers with lengths <10 µ and/or diameters >1.0 µ by fiber type.

Dimensions	Amosite	Crocidolite	Tremolite	Actinolite	Anthophyllite	Chrysotile	LA
<10 µ L	9	12	42	41	13	35	12.5
>1.0 µ D	1	1	36	23	39	23	31
Both	0	1	10	14	0	3	3

*L = fiber length; D = fiber diameter; LA = Libby amphibole

sides may nonetheless be cleavage fragments with little or no pathogenic effects. The vast majority of asbestos bodies are formed on commercial amphibole (amosite or crocidolite) cores.

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